PATENT
Attorney Docket No.: 015280-196310US
Client Ref. No.: E-054-94/2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Raoul E. Benveniste, et al.

Application No.: 09/769,223

Filed: January 24, 2001

For: METHOD OF INDUCING CELL-MEDIATED PROTECTIVE IMMUNITY AGAINST HIV USING LOW DOSES OF IMMUNOGENS

Customer No.: 20350

Confirmation No.

Examiner: Jeffrey S. Parkin

Technology Center/Art Unit: 1648

AFFIDAVIT UNDER 37 C.F.R. §1.131

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Dr. Raoul E. Benveniste, being duly warned that willful false statements and the like are punishable by fine or imprisonment or both, under 18 U.S.C. §1001, and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. All statements herein made of my own knowledge are true and statements made on information or belief are believed to be true.

2. I am the named lead inventor of U.S. Patent Application No. 09/769,223.

3. With respect to the professional qualifications, experience and knowledge which underly this affidavit:

I received my A.B. degree in 1966 with honors from The Johns Hopkins University; an M.S. degree in 1970 in Biochemistry from the University of Wisconsin; a Ph.D. degree in 1971 in Molecular Biology from the University of Wisconsin; and an M.D. degree in 1979 from the University of Miami School of Medicine.

During 1979-1980, I was an Intern in Medicine at The Johns Hopkins Hospital Osler Medical Service, Baltimore, MD.

From 1999 to the present I have been employed as a Medical Officer of the NIH, NCI, Center for Cancer Research, Frederick MD. From 1981-1999, during the time when the invention was made, I was a Medical Officer, with the NIH, NCI, DBS, Laboratory of Viral Carcinogenesis in Bethesda, MD and Frederick, MD. From 1971-1974, I was a Staff Fellow, NIH, NCI, DCE, Laboratory of Viral Carcinogenesis, Viral Leukemia and Lymphoma Branch, Bethesda, MD. From 1974-1977, I was a Senior Staff Fellow, NIH, NCI, DCE, Laboratory of Viral Carcinogenesis, Bethesda, MD.

I have served on the following Scientific Advisory Committees:

1975-1977 -	NIH Primate Utilization Review Group
1975-1977 -	Project Officer for studies of tumor viruses in nonhuman primates at Rush-Presbyterian - St. Luke's Medical Center, Chicago, IL
1988-1991 -	NCI AIDS Vaccine Task Force
1997-2000 -	Chairman, NCI Intramural Primate Advisory Committee

My areas of primary research interest include the molecular biology and evolution of retroviruses, the role of viral genes in disease, and the SIV - macaque model for development of AIDS vaccines and antiviral compounds. As set forth in the accompanying bibliography (Appendix A), I have extensively published in each of these research areas (see particularly references 60-138).

4. I understand that the Examiner has raised a concern regarding the operability of the subject matter of claims 17, 31, 32 and 40-44. This affidavit principally concerns two issues identified by the Examiner: a) the predictive value of the macaque simian immunodeficiency virus model for HIV in the human and b) the ability of a low-dose HIV or SIV immunogens to induce a cell-mediated immune response without generating a noticeable humoral response.

a) The Predictive Value of the Macaque Simian Immunodeficiency Model for Human HIV

The Simian Immunodeficiency Virus (SIV) model serves an excellent model for HIV disease and pathogenesis. Vaccine studies in the macaque have predicted human vaccine trial failures, and have pointed the way to successful approaches just now being initiated in human trials. In fact, previous data, using the SIV model, predicted the failure of the HIV-1 phase III trial that was completed this past summer. That trial (VaxGen) used gp120 surface protein as an immunogen, and previously published SIV vaccine research had shown that the gp120 immunogen does not confer protection from an infectious virus challenge.

The time to death due to AIDS in the SIV macaque model and in humans infected with HIV is very similar. For example, HIV infection in humans causes death in untreated individuals in approximately 10 -12 years (about 15% of our lifespan). SIV/Mne results in CD4+ cell depletion in about 20 to 33 months (depending on macaque species), and death due to AIDS in 26 to 36 months. Since macaque lifespan is approximately 20 years, SIV/Mne leads to death in macaques in about 11% to 15% of their lifespan as well, and should serve as a good model for proof-of-concept vaccine studies in macaques.

SIV and HIV infect the same subset of cells (CD4+ lymphocytes, dendritic cells, etc) in their respective hosts, and lead to CD4+ cell depletion and opportunistic infections.

Viral load in plasma present a few weeks after initial infection is predictive, both in humans and macaques, of the rapidity of the disease progression. Peak viral loads are similar in both systems.

Infection of pregnant macaques during the second or third trimester results in approximately 25% to 33% of newborns infected with SIV. This is similar to the percentage of infected human infants born to untreated HIV positive mothers. Transmission of SIV or HIV via breast milk has been shown to occur both in human and macaque newborns.

Authorities in the field support the value of the SIV macaque model as an excellent model for HIV disease in pathogenesis as evidenced in numerous publications, including the 5 publications set forth in Appendix B and discussed below:

1. **Polacino PS, Stallard V, Klaniecki JE, Pennathur S, Montefiori DC, Langlois AJ, Richardson BA, Morton, WR, Benveniste RE, Hu S-L. Role of Immune Responses against the Envelope and the Core Antigens of Simian Immunodeficiency Virus SIVmne in Protection against Homologous Cloned and Uncloned Virus Challenge in Macaques. J. Virol. 1999. 73: 8201-15.**

This paper systematically addresses which regions of SIV are needed to elicit a protective response against an intravenous challenge with both homologous and heterologous SIV viruses. Immunization involved priming with vaccinia virus recombined with and expressing various SIV proteins and then boosting with the corresponding proteins (prime and boost immunizations). The results clearly show that a vaccine consisting of just envelope surface protein (gp120) was not sufficient to protect against a heterologous virus challenge; the addition of the transmembrane envelope protein (gp32) was essential to elicit protection from infection. This result was predictive of the failure of the recently completed VaxGen phase III human AIDS vaccine trial, which showed that a vaccine consisting of just gp120 did not protect from AIDS virus infections.

2. Hirsch VM, Lifson JD. Simian immunodeficiency virus infection of monkeys as a model system for the study of AIDS pathogenesis, treatment, and prevention. Adv. Pharmacol. 2000. 49: 437-77.

This review summarizes the SIV model system and its relevance in studying the human disease. The authors are both highly respected in the AIDS field: Dr. Vanessa Hirsch is chief of a laboratory at NIH (NIAID) and Dr. Lifson is director of the AIDS Vaccine Program at NCI-Frederick. My bibliography (Appendix A) shows that Dr. Lifson and I have collaborated on several manuscripts.

The authors state: "... represents a tremendous resource, among other things allowing the investigator to select the system that best recapitulates particular aspects of human HIV infection for study in a relevant nonhuman primate model. Such studies have provided, and may

be expected to continue to provide, important insights to guide HIV treatment and vaccine development in the future."

3. Geretti AM. Simian immunodeficiency virus as a model of human disease. Rev. Med. Virol. 1999. 9: 57-67.

The author states: "Because of strong clinical, pathological, virological and immunological analogies with HIV infection of humans, infection of macaques with SIV provides a valuable model for exploring crucial issues related to both the pathogenesis and prevention of HIV infection. This model has offered a unique setting for the preclinical evaluation of drugs, vaccines and gene-therapies against HIV, and has helped to identify many viruses and host determinants of lentiviral disease."

4. Zink MC, Spelman JP, Robinson RB, Clements JE. SIV infection of macaques – modeling the progression to AIDS dementia. J. Neurovirol. 1998. 4: 249-259.

Dr. Clements is a professor and chairman at The Johns Hopkins Medical School. The authors state: "The SIV/macaque model is excellent for the study of viral virulence factors and host responses to infection. This review outlines how the SIV/macaque model has been used to identify viral factors that are important for the development of neurological disease, to determine when HIV enters the brain, and to characterize the host immune responses affecting virus entry to the CNS and the development of neurological disease."

5. McClure HM, Anderson DC, Ansari AA, Klumpp SA. The simian immunodeficiency virus infected macaque: a model for pediatric AIDS. Pathol Biol (Paris) 1992. 40: 694-700.

The authors, who are affiliated with the Yerkes National Primate Research Center in Atlanta, Georgia, state: "The SIV-infected macaque should prove to be a useful model to evaluate the timing and mechanisms of lentivirus infection in infants, to determine maternal factors associated with transmission to the fetus or infant, and to evaluate therapeutic regimens for the prevention or treatment of pediatric AIDS."

b) Ability of Low Dose Immunogens to Induce a Cell-mediated but not a Humoral Response

In collaboration with others, I first reported that T-cell proliferation to subinfectious SIV correlates with lack of infection after challenge of macaques in 1994 (see, Appendix C, Clerici M, Clark EA, Polacino P, Axberg I, Kuller L, Casey NI, Morton WR, Shearer GM, Benveniste RE. AIDS. 1994 Oct;8(10):1391-5.)

We analyzed correlates of protection in macaques exposed to SIV. Peripheral blood mononuclear cells (PBMC) from macaques inoculated intrarectally with various dilutions of SIV were examined for their *in vitro* proliferative response to SIV envelope peptides and generation of SIV-specific antibodies. The viral-specific immune responses of macaques exposed to infectious doses of SIV were characterized by generation of antibodies and weak or undetectable T-cell-mediated responses. In contrast, macaques inoculated with doses of SIV below the threshold required for seroconversion and recovery of virus (these doses ranged from 0.1 animal infectious doses (AID) to 0.0001 AID of the immunogen) exhibited T-cell proliferation in response to SIV envelope synthetic peptides. Some macaques previously exposed intravenously to subinfectious doses of SIV were subsequently challenged 16 months later with an infectious intrarectal dose of SIV. Macaques that had previously been exposed to 0.1 AID and 0.001 AID of SIV resisted the subsequent virus challenge, whereas the naive macaques (never exposed to SIV) all became infected. The inability to productively infect macaques previously exposed to subinfectious doses of SIV suggested that a T-cell-mediated response (in the absence of a detectable humoral response) may confer long-term protection against infection, and that AIDS vaccines should be designed to optimize the cellular arm of the immune response. Thereafter, several investigators have shown protection after immunizing macaques with sub-infectious or low doses of virus (the references are found in Appendix D):

1. Putkonen P, Makitalo B, Bottiger D, Biberfeld G, Thorstensson R. Protection of human immunodeficiency virus type-2 exposed seronegative macaques from mucosal simian immunodeficiency virus transmission. J. Virol. 1997. 71: 4981-4.

This manuscript shows that two macaques exposed to subinfectious doses of HIV-2 developed SIV-specific cytotoxic T lymphocytes, and were protected from a subsequent intrarectal (mucosal) challenge with infectious SIV. The authors state: "Here we provide evidence that activation of the cell-mediated arm of the immune system only, without antibody

formation, can control SIV replication in macaques. The results imply that vaccines that stimulate a strong and broad cellular immune response could prevent mucosal HIV transmission."

2. Murphey-Corb M, Wilson LA, Trichel AM, Roberts DE, Xu K, Ohkawa S, Woodson B, Bohm R, Blanchard J. Selective induction of protective MHC class I-restricted CTL in the intestinal lamina propria of rhesus monkeys by transient SIV infection of the colonic mucosa. J. Immunol. 199. 162: 540-9.

This paper reveals that two rhesus macaques had no signs of infection in the jejunum, mesenteric lymph nodes and peripheral blood mononuclear cells (PBMC) following colonic exposure to molecular clones of SIV. Three or six months postexposure, these animals and naïve controls were challenged intracolonically with the heterologous primary isolate, SIV/DeltaB670. All macaques with strong SIV env-specific MHC-restricted CTL in the lamina propria (LP) of the jejunum were protected. The authors state: "The identification of mucosal immune responses required for protection against sexual transmission of HIV is essential for the development of an efficacious vaccine." "Furthermore, a strong correlation between SIV env-specific MHC-restricted CTL in the LP and protection against colonic mucosal challenge was observed."

3. Wilson LA, Murphey-Corb M, Martin LN, Harrison RM, Ratterree MS, Bohm RP. Identification of SIV env-specific CTL in the jejunal mucosa in vaginally exposed, seronegative rhesus macaques (Macaca mulatta). J. Med. Primatol. 2000. 29: 173-81.

This manuscript shows the env-specific CTL were induced in jejunal LP in five of eight non-progesterone treated macaques that were vaginally (mucosally) exposed to SIV, but not infected. There was delayed disease progression and lower viral loads in plasma observed only in those macaques exhibiting jejeunal env-specific CTL. The authors state: "Those monkeys with strong mucosal CTL responses specific for ...SIV envelope (env) were protected from later colonic challenge with a heterologous pathogenic virus dose." "The association of such CTL with protection or delayed disease upon challenge suggests that successful vaccine protection against SIV/HIV may require CTL responses in the mucosa."

The human data on the presence of cell-mediated immunity in HIV-1 resistant individuals has been published previously and is in accord with the role of CTL in the macaque. Three such references are found in Appendix E:

1. SL Rowland-Jones et al. HIV-specific cytotoxic T-cell activity in an HIV-exposed but uninfected infant. Lancet 341: 860-861, 1993.

In accord with its title, this reference disclosed a HIV specific cytotoxic T-cell activity to be associated with non-infection in a HIV-exposed infant.

2. SL Rowland-Jones et al. HIV-specific cytotoxic T-cells in HIV-exposed but uninfected Gambian women. Nature Medicine 1: 59-64, 1995.

This key paper states that the CTL activity in these HIV-exposed but uninfected women may represent protective immunity against HIV infection. As set forth therein:

> A crucial requirement in the rational design of a prophylactic vaccine against the human immunodeficiency virus (HIV) is to establish whether or not protective immunity can occur following natural infection. The immune response to HIV infection is characterized by very vigorous HIV-specific cytotoxic T-lymphocyte (CTL) activity. We have identified four HIV-1 and HIV-2 cross-reactive peptide epitopes, presented to CTL from HIV-infected Gambians by HLA-B35 (the most common Gambian class I HLA molecule). These peptides were used to elicit HIV-specific CTLs from three out of six repeatedly exposed but HIV-seronegative female prostitutes with HLA-B35. These women remain seronegative with no evidence of HIV infection by polymerase chain reaction or viral culture. Their CTL activity may represent protective immunity against HIV infection.

3. **Rowland-Jones SL, Dong T, Dorrell L, Ogg G, Hansasuta P, Krausa P, Kimani J, Sabally S, Ariyoshi K, Oyugi J, MacDonald KS, Bwayo J, Whittle H, Plummer FA, McMichael AJ Broadly cross-reactive HIV-specific cytotoxic T-lymphocytes in highly-exposed persistently seronegative donors. Immunol Lett. 1999 Mar;66(1-3):9-14.**

As set forth therein:

> HIV-specific cytotoxic T-lymphocytes (CTL) are believed to play a key part in the control of virus levels throughout HIV infection. An important goal of a potential prophylactic vaccine against HIV is therefore to elicit a strong CTL response which is broadly cross-reactive against a diverse range of HIV strains. We have detected HIV-specific CTL in two groups of highly-exposed but persistently seronegative female sex workers in Africa which show extensive cross-reactivity

> between different viral sequences. In a small group of women exposed to both HIV-1 and HIV-2 in Gambia, studied over 4 years, we have repeatedly detected HLA-B35-restricted CTL which exhibit cross-reactivity between the HIV-1 and HIV-2 sequences of the CTL epitopes. In women with particularly intense exposure to what are likely to be multiple clades of HIV-1 in Nairobi Kenya, we have detected CTL directed towards epitopes conserved between HIV-1 clades. In neither group is there any evidence that variation in CCR5 sequence or expression is responsible for their apparent resistance to HIV infection. However, in seropositive donors from Oxford infected with African strains of HIV-1, we have defined CTL responses which are specific for particular clades and have mapped some unique A clade CTL epitopes, together with others to highly-conserved regions of the virus. Further information about the extent of cross-reactive CTL immunity will be important for future vaccine design and evaluation.

The observations that the SIV macaque model is a useful model for HIV infection in the human, that in the macaque SIV model low dose (and subinfectious) immunogens can confer a cytotoxic T-cell mediated resistance to SIV infection, and the association of a cytotoxic T-cell response to HIV with human resistance to HIV infection or progression, strongly support the operability of the claimed subject matter.

This Declarant has nothing further to say.

Raoul E. Benveniste

Raoul E. Benveniste, Ph.D., M.D.

April 1, 2004

Date

60180619 v1